

F4 acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, and wherein the *cis*-acting nucleotide sequence does not comprise a full-length coding region.

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- F5
3. The *cis*-acting nucleotide sequence according to claim 1 derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$  3'-UTR).
  4. The *cis*-acting nucleotide sequence according to claim 3 which comprises:
    - a) the nucleotide sequence substantially as denoted by SEQ ID NO:1; or
    - b) biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1; or
    - c) a nucleotide sequence whose complementary nucleotide sequence hybridizes, under conditions which allow for such hybridization to occur, with the nucleotide sequences of (a) or (b).
  5. The *cis*-acting nucleotide sequence according to claim 4, which comprises:
    - a) the nucleotide sequence as denoted by SEQ ID NO:2; or
    - b) biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:2; or
    - c) a nucleotide sequence whose complementary nucleotide sequence hybridizes, under conditions which allow for such hybridization to occur, with the nucleotide sequences of (a) or (b).
  6. The *cis*-acting nucleotide sequence according to claim 5 wherein said gene encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, protein which is an agricultural product, and a protein which is an industrially applicable product.

7. A DNA construct comprising:-
- a gene which contains at least one intron;
  - a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and
  - optionally further comprising additional control, promoting and/or regulatory elements.
- FS 8. The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence comprises:
- the nucleotide sequence as denoted by SEQ ID NO:1; or
  - biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1; or
  - a nucleotide sequence whose complementary sequence hybridizes, under conditions which allow hybridization to occur, with the nucleotide sequences of (a) or (b).
9. The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence comprises:
- the nucleotide sequence as denoted by SEQ ID NO:2; or
  - biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:2; or
  - a nucleotide sequence whose complementary sequence hybridizes, under conditions which allow for such hybridization to occur, with the nucleotide sequences of (a) or (b).
10. A DNA construct according to any one of claims 7 to 9 wherein said control, promoting

and/or regulatory elements are suitable transcription promoters, transcription enhancers and mRNA destabilizing elements.

11. The DNA construct according to claim 7, wherein said gene which contains at least one intron, encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, protein which is an agricultural product, and a protein which is an industrially applicable product.
12. The DNA construct according to claim 11 wherein said nucleotide sequence is contained within an exon of said gene.
13. The DNA construct according to claim 11 wherein said nucleotide sequence is contained within an intron of said gene.
14. The DNA construct according to claim 13 wherein said gene is the human TNF- $\alpha$  gene.
15. The DNA construct according to claim 14 being the plasmid pTNF- $\alpha$ , in which said *cis*-acting element is contained within an exon of the human TNF- $\alpha$  gene.
16. The DNA construct according to claim 15 being the plasmid pTNF- $\alpha$ (3'UTR- $\alpha$ EP).
17. The DNA construct according to claim 7 wherein said gene is the human TNF- $\beta$  gene.
18. The DNA construct according to claim 17 in which said *cis*-acting element is contained within an exon of the human TNF- $\beta$  gene.
19. The DNA construct according to claim 18 being the plasmid pTNF- $\beta$   
(3'UTR- $\alpha$ ).
20. The DNA construct according to claim 18 being the plasmid pTNF- $\beta$ (3'UTR- $\alpha$ EP).
21. The DNA construct according to claim 14 in which said gene is the human TNF- $\alpha$  gene and said *cis*-acting element is contained within an intron of said gene.

22. The DNA construct according to claim 21 being the plasmid pTNF $\alpha$ ( $\Delta$ 3'UTR)i3EP.
23. A vector comprising a *cis*-acting nucleotide sequence according to claim 1 or a DNA construct according to claim 7 and a suitable DNA carrier, capable of transfecting a host cell with said *cis*-acting nucleotide sequence.
24. The vector according to claim 23 optionally further comprising additional expression, control, promoting and/or regulatory elements operably linked thereto.
25. The vector according to claim 24 wherein said carrier is salmon sperm DNA.
26. The vector according to claim 24 wherein said carrier is viral DNA.
27. The host cell transfected with a DNA construct according to claim 22.
28. A host cell transfected with a vector according to claim 23.
29. A host cell according to claim 27 or 28 being a eukaryotic or yeast cell.
30. The host cell according to claim 29 being a mammalian hemopoietic cell, fibroblast, epithelial cell, or lymphocyte.
31. The host cell according to claim 27 wherein said eukaryotic cell is the baby hamster kidney (BHK-21) cell line or the Chinese hamster ovary (CHO) cell line.
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47. A pharmaceutical composition comprising as active ingredient a therapeutically effective amount of expression vectors according to any one of claims 23 to 26 or of transformed host cells according to any one of claims 30 and 31.
48. A method of producing a recombinant therapeutic or industrially or agriculturally applicable protein comprising the steps of:
- a) providing a DNA construct according to claim 7 or an expression vector according to claim 23 wherein said gene encodes said protein;
  - b) transfecting a host cell with a DNA construct or expression vector provided in (a) to

give a host cell capable of expressing said protein in substantial amount; and

- c) culturing cells obtained in (b) under suitable culture conditions; and
- d) isolating said protein from the cell culture obtained in (c).

49. A method of producing a recombinant therapeutic or industrially or agriculturally applicable protein comprising the steps of:

- a) providing host cells transfected with a DNA construct according to claim 7 or an expression vector according to claim 23 wherein said gene encodes said protein, which are capable of expressing said protein in substantial amount;
- b) culturing cells provided in (a) under suitable culture conditions; and
- c) isolating said protein from the cell culture obtained in (b).

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### REMARKS

Claims 1-31 and 47-49 are pending. Claim 2 has been cancelled by this communication. Claims 1, 3-32 and 47-49 have been amended to more clearly indicate the subject matter which Applicants regard as the invention. No new matter has been introduced by the amendment to the claims.

In accordance with 37 C.F.R. §1.121, applicants have provided (1) accurate instructions to amend the claims, (2) replacement claims in clean form herein, and (3) another version of the amended claims marked up to show all the changes relative to the previous version of the claims, which appears on an attached page.

#### I. Information Disclosure Statement Objections and Drawings

The Examiner has indicated that (1) the patent documents in the information disclosure statement filed on June 12, 2001 will not be considered because they are duplicates of the documents in the IDS filed on September 4, 2001, (2) the information disclosure statement